Preparation of Matrix DNA Chip and Labeling of DNA Hybridization by Electrochemical Method

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It is known that P-aminothiophenol (PATP) is easy to self-assemble on Au surface to form an order monolayer[1]. In this research work, a PATP self-assemblized Au base plane is used to immobilize a synthesized 20-mer single-stranded dexoyribonucleic acid (ss-DNA) and the hybridization of ss-DNA with its complementary ss-DNA is electrochemically labeled by the redox current of a organic sulf-compound. Meanwhile, a method to prepare matrix DNA chip is proposed.

PATP was self-assembled on Au surface by the method described in reference [2]. Then the PATP/Au was immersed in 0.1M 2-(N-morpholino0ethanesulfonic acid (MES) buffer (pH6.0) containing 0.165mg/ML ss-DNA and 0.1M 1-ethy-3(3-dimethylaminopropyl)-carbodiimide (EDC) and heated in a water bath at 45¡æ for 3h. Therefore a synthesized 20-mer ss-DNA with the sequence of 5'-TGC AGT TCC GGT GGC TGA TC-3' was immobilized on the order monolayer PATP by a phosphoramidate bond between the 5'-terminal phosphate groups of ss-DNA and the amine groups on the PATP under the presence of EDC[3]. The immobilization of ss-DNA on PATP/Au electrode was proved by the diffuse reflectance IR spectra (shown in Fig.1) and by the cyclic voltammograms(CVs) of 10μM $Co(phen)_3^{2+}$ on the ss-DNA/PATP/Au electrode in Tris buffer solution. After that, the ss-DNA immobilized on order momolayer PATP/Au was hybridized with complementary ss-DNA in a hybridization buffer solution containing 0.07μg/ML complementary ss-DNA, the solution was shaking in a water bath at 59¡æ for 1.5h.

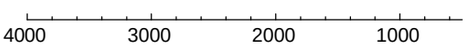

Fig.1 The diffuse reflectance IR spectrum of ss-DNA on the order monolayer PATP/Au.

Fig.2 shows the CVs of 5×10^{-5}M organic sulf-compound at ss-DNA/PATP/Au electrode and ds-DNA/PATP/Au electrode in a Tris buffer solution (pH7.0). It is obviously that the redox current of organic sulf-compound is almost disappear after hybridization, and demonstrates that this organic sulf-compound will be a good indicator for the electrochemically labeling of the hybridization process of DNA. The interactions between organic sulf-compound with ds-DNA and that between $Co(phen)_3^{2+}$ with ds-DNA were compared by some other CV experiments, and showed that organic sulf-compound has stronger interaction with ds-DNA than $Co(phen)_3^{2+}$. The result tends to consider that organic sulf-compound is intercalated completely into the chain of ds-DNA and therefore loses its redox activity.

At the same time, a nano-Au array was prepared through an anodic aluminum oxide template by using some special method, then ss-DNA was immobilized on the nano-Au array by the above method, and a high density matrix DNA chip was also obtained.

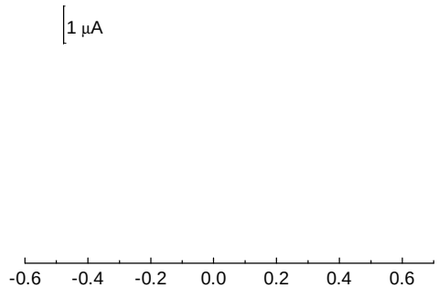

Fig.2 Cyclic voltammograms of 5×10^{-5}M organic sulf-compound at ss-DNA/PATP/Au electrode(1) and ds-DNA/PATP/Au electrode(2) in 10mM Tris buffer solution(pH7).

The above DNA chips will improve the preparation and application of DNA chip because of its high density and its high order of DNA.

The study of the detailed interaction mechanism of organic sulf-compound with ds-DNA and the ripe preparation and application of the matrix DNA chip are still going on.

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